

CLAIMS

That which is claimed is:

1. A method of hydrogen gas generation, comprising the steps of:
culturing algae under illuminated conditions in a media comprising sulfur wherein sulfate permease expression of the algae is reduced relative to normal wild-type algae;
sealing the algae culture from atmospheric oxygen; and
collecting hydrogen gas evolved.
2. The method of claim 1, wherein the algae is a green algae and the algae comprises a genome which is artificially engineered to reduce sulfate permease expression relative to a wild-type algae.
3. The method of claim 2, wherein the algae is a unicellular, photosynthetic, anoxygenic algae.
4. The method of claim 1, wherein the algae is chosen from *Rhodobacter sphaeroide* and genetically modified *Chlamydomonas reinhardtii*.
5. The method of claim 1, wherein the algae is *Rhodobacter sphaeroide* an anoxygenic photosynthesis bacterium having a lineage of *Proteobacteria*; *alphaproteobacteria*, *Rhodobacterales*; *Rhodobacteraceae*.
6. The method of claim 1, wherein the algae is an isolated strain with downregulated expression of sulfate permease with 50% or less expression of sulfate permease relative to normal wild-type algae.
7. The method of claim 2, wherein the algae is genetically modified by insertion of an antisense sequence to *CrcpSulP*.
8. The method of claim 2, wherein the genetically-modified algae is modified by a technique chosen from insertion of an antisense strand of *CrcpSulP*, insertion of a sense strand of *CrcpSulP*, ablation of *CrcpSulP* and targeted gene deletion of *CrcpSulP*.

9. The method of claim 7, wherein the antisense sequence hybridizes to a portion of SEQ ID NO:2.
10. An isolated nucleotide sequence, chosen from SEQ ID NO:2; SEQ ID NO:3; SEQ ID NO:4, SEQ ID NO:5; SEQ ID NO:6 and a sequence which hybridizes to any one of SEQ ID NO:2 and SEQ ID NO:3; SEQ ID NO:4, SEQ ID NO:5; SEQ ID NO:6.
11. An isolated amino acid sequence selected from the group consisting of SEQ ID NO:1 and a sequence with 90% or more sequence homology to SEQ ID NO:1.
12. A genetically-modified algae wherein the sulfate uptake pathway is downregulated to 50% or less relative to a native, wild-type, unmodified algae.
13. The algae of claim 12, wherein the alga is a green algae.
14. The algae of claim 13, wherein expression of an endogenous *CrcpSulP* gene is downregulated by insertion of an antisense *CrcpSulP* polynucleotide into the genome of the algae.
15. The algae of claim 14, wherein the algae is *Chlamydomonas reinhardtii*.
16. The algae of claim 12, wherein the expression of the *CrcpSulP* gene is downregulated by an antisense sequence that hybridizes to a portion of the *CrcpSulP* mRNA transcript.
17. A composition, comprising:
water;
algae growth nutrients;
algae genetically modified for sulfate permease expression reduced by 50% or more relative to an unmodified wild-type version of the algae.
18. The composition of claim 17, wherein the algae is unicellular, photosynthetic, anoxygenic algae.
19. An assay for detecting low levels of sulfur uptake in a sample of genetically-modified green algae comprising the steps of:

- a. culturing a genetically-modified sample of green algae in TAP media in lighted, anaerobic conditions;
- b. transferring an aliquot of the sample into a media comprising sulfur;
- c. culturing the aliquot in lighted conditions; and
- d. detecting the level of ARS activity in the aliquot,

wherein an elevated level of aryl-sulfatase (ARS) activity is a positive indicator that the genetically-modified green algae is deficient in sulfur uptake compared to a wild-type algae.

20. An isolated antisense oligonucleotide consisting of a nucleotide sequence that is complementary to SEQ ID NO:2.
21. An isolated antisense oligonucleotide comprising a sequence complementary to codons 118 to 412 of SEQ ID NO 2.
22. An expression vector comprising an antisense sequence complementary to codons 118 to 412 of SEQ ID NO:2.
23. A composition, comprising:
a *sulP1* strain of *Chlamydomonas reinhardtii*; and
a *Rhodobacter sphaeroides* bacterium that is anaerobic and photosynthetic.
24. The composition of claim 23, further comprising a *Clostridium* sp having the lineage *Bacteria; Firmicutes; Clostridia; Clostridiales; Clostridiaceae*.
25. A process for producing hydrogen comprising culturing a combination of *sulP1* strain of *Chlamydomonas reinhardtii* and *Rhodobacter sphaeroides* with *Clostridruim* sp.
26. A method of generating hydrogen gas, comprising the steps of:
providing in an aqueous media a *sulP1* strain of *Chlamydomonas reinhardtii* and *Rhodobacter sphaeroides* bacteria;
exposing the aqueous media to sunlight for a period of time and under conditions to allow for the generation of hydrogen.
27. The method of claim 26, further comprising:
providing *Clostridium* in the media.

28. A method for generating hydrogen gas, comprising the steps of:
 - subjecting a biomass comprising an algae to sunlight in a sulfur-containing media comprising carbon dioxide and inorganic nutrients for a period of time and under conditions so as to cause the algae to undergo oxygenic photosynthesis and to generate hydrogen gas; and
 - subjecting an anaerobic photosynthetic bacterium in the media to sunlight for a period of time and under conditions so as to generate hydrogen from a nitrogenase/hydrogenase enzymatic system in the media.
29. The process of claim 28, further comprising:
 - inducing fermentation of the biomass the media of *Chlamydomonas/Rhodobacter* via *Clostridium sp.*
30. A method of generating hydrogen gas, comprising the steps of:
 - providing in an aqueous media a genetically-modified strain of *Chlamydomonas reinhardtii*
 - providing a strain of *Rhodobacter sphaeroides* photosynthetic bacteria;
 - exposing the aqueous media to sunlight for a period of time and under conditions to allow for the generation of biomass and hydrogen;
 - subjecting an anaerobic photosynthetic bacterium in the media to sunlight for a period of time and under conditions so as to generate hydrogen from a nitrogenase/hydrogenase enzymatic system in the media;
 - providing a strain of *Clostridium* in the media; and
 - inducing fermentation of the biomass in the media via *Clostridium sp.*
31. The method of claim 30, wherein the genetically-modified algae is modified to decrease activity of sulfate permease by a technique selected from the group consisting of insertion of an antisense strand of a sulfate permease gene, insertion of a sense strand of a sulfate permease gene, ablation of the sulfate permease gene and targeted gene deletion of the sulfate permease gene.